C2 Biotechnologies, LLC

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SOP-112

Bacterial Glycerol Stocks

Procedures used to produce bacterial glycerol master and working stocks.

Section 1

Materials and Reagents

Glycerol

Bacterial Culture

Sterile cryo-vial

Section 2

Protocol

Purpose:

Bacterial strains may be stored indefinitely at low temperatures (- 20 degrees C and -80 degrees C) in 15 to 40% glycerol. It is general laboratory policy to prepare a frozen stock of newly acquired or created strains for repository. Two repositories are generated: 1, Master Repository and 2, Working Repository. The Master Repository or Master Stock contains a copy of all strains used in the laboratory for long term storage and back up. The Working Repository or Working Stock contains all strains in the laboratory and is used for routine laboratory operations.

Time required:

18 hours. Bacterial cells are grown overnight and used to generate a glycerol stock(s).

Procedure:

Day 1

1. Inoculate a 5 ml culture tube containing 5 ml of LB media or LB media+antibiotic with a freshly grown isolated colony. Incubate at 37 C with shaking overnight.

Day 2

1. For each strain to be stored at -80 C prepare and label sterile cryo-vial. The label must contain name of culture, media used, date and initials of preparer.

   Pipet 225 ul sterile 80% glycerol into the cryo-vial. Add 1.0 ml of the bacterial culture (15% glycerol final concentration). Mix well (vortex) and place tube at -80 C.

2. Complete repository log information sheet.

To recover a strain from the -80 C glycerol stock use a sterile pipette to scrape some of the ice then streak out the cells on the appropriate medium e.g. LB media + ampicillin.

Note: Do not allow frozen stock to thaw because each freeze-thaw cycle will result in a 50% loss in cell viability.

Nomenclature:

When at all possible the naming of plasmids will conform to rules developed by Novick *et al.* (1976).

Generally for our purposes a parental plasmid will be modified by an individual. In this case the plasmid may be named using the initials of the individual preceded by a lower case p and followed with a numerical value. The numerical value may be the series in which the construct was generated. The genotype and phenotype description of the construct will follow the rules set by Novick *et al.* (1976). The Master and Working Stock log will provide a human readable description of the construct.

For example we use pHT43 as our base expression vector. This vector is commercially available and was generated by Phan Thi Phuong Trang which is a hybrid or derivative of pNDH37 with the deletion of a 117-bp direct repeat and is the 43 in a series of constructs. If Dr. Larry Cosenza inserts gene *tva* which produced the protein phenotype Tva into pHT43 and it is his first construct it may be labeled pLC100. The genotype and phenotype description will indicate that pHT43 was the parental vector and what mutational work LC performed.

For glycerol stocks the bacterial name and or strain is given followed by the plasmid name enclosed in parenthesizes. So for B. subtilis strain WB800N carrying pLC100 the cryo-vial maybe labeled *B. subtilis* WB800N(pLC100)

Example Repository Log Sheet

Freezer_____Title_____

Shelf_____ PI_____

Section 3

References

Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999

Novick RP, Clowes RC, Cohen SN, Curtiss R 3rd, Datta N, Falkow S. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol Rev. 1976 Mar;40(1):168-89